CELLULAR ANTIGENS IN THE STRUCTURE OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS

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CELLULAR ANTIGENS IN THE STRUCTURE OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS

P. N. Kosyakov, T. A. Posevaya, T. M. Sokolova, O. V. Zaitseva, L. K. Menshikh, F. I. Ershov

Venezuelan equine encephalomyelitis virus was found to incorporate into its structure antigens of the cells in which it multiplies. A highly purified virus grown in chick fibroblast cultures was found to have the species-specific antigen, heterogenous Forssman antigen and group A and H antigens. These antigens are localized differently in the virus with the species antigen on the surface, group antigens and heterogenous Forssman antigen localized deeper and are inaccessible to the effect of specific antibody in native virus. These antigens are detected only after destruction of virions with different detergents. The mechanism of incorporation of these antigens into the virus structure is discussed.

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CELLULAR ANTIGENS IN THE STRUCTURE OF VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS

Article by P. N. Kosyakov, T. A. Posevaya, T. M. Sokolova, O. V. Zaytseva, L. K. Men'shikh and F. I. Yershov in the Russian-language journal Vop. Virusol. Problems of Virology 19:4:455-459, 1974, submitted 6 August 1973, Institute of Virology imeni D. I. Ivanovskiy of the USSR Academy of Medical Sciences, Moscow.

It was established that the Venezuelan Equine Encephalomyelitis (VEE) virus incorporates into its structures antigens of the cells in which it multiplies. A highly purified virus grown in chick fibroblast cultures was found to have the species-specific antigen, heterogeneous Forssman antigen and group A and H antigens. These antigens are localized differently in the virus with the species antigen on the surface, group antigens and heterogeneous Forssman antigen localized deeper and inaccessible to the effect of specific antibodies in the native virus. These antigens are detected only after destruction of virions with various detergents. The paper deals with the mechanism of incorporation of these antigens into the virus structure.

It was established that the viruses of influenza (3, 10, 11) parainfluenza (Sendai), (4), fowl plague, both true and false, (2), smallpox vaccine (7) contain in the structure of the tunica adventitia antigens of cells on which these viruses were cultivated. The ability to

incorporate into their structures antigens of cells is not inherent in all viruses according to our studies (5). Thus, we did not find antigen cell components in virus ECHO 12.

The present study was designed to investigate one of the arbovirus representatives for the presence in its composition of antigens in the host cell.

Materials and Methods

The subject of our study was the VEE virus, SPF strain which was passivated in an initially trypsinated culture of chick fibroblasts in medium 199 containing 2 % bovine serum.

Purification and Concentration of the Virus

The virus containing the liquid culture medium (infectious titre 8-9 lg BOE/ml) was purified (10,000 rpm for 30 min's) and virus sedimentation was achieved by centrifugation (35,000 rpm for 90 min's, rotor 8 x 50 ml, MSE-65 Centrifuge). The viral sediment was resuspended in a standard tris-buffer (0.01 M tris 0.1 M NaCl, 10 mM ethylenediaminetetraacetic acid sodium salt 'EDTA') and subjected to purification in a graduated saccharose density gradient of 20-60 % (25,000 rpm for 2 hrs, rotor 3 x 25 ml, MSE-65 Centrifuge). Following centrifugation we removed the interphase containing the virus, diluted it and precipitated the virus in the precipitate through 15 % saccharose (45,000 rpm for 90 min's, rotor 10 x 10 ml, MSE-65 Centrifuge). Purification of the virus for protein at this stage was 99.95 %, the infectious titre of the purified virus in comparison with the original increased approximately by 2 lg BOE/ml (8).

In order to obtain a homogeneous viral preparation it was fractionated in a linear 15-40 % saccharose gradient (25,000 rpm for

90 min's, rotor 3 x 25 ml, MSE-65). The gradient was separated in the collector and fractions were removed containing the maximum absorption at 260 nm, infection and radioactivity. The saccharose was removed by precipitating the virus in the centrifuge (45,000 rpm for 90 min's, rotor 10 x 10 ml, MSE-65). The virus suspension was resuspended in a standard tris-buffer and was used for immunological analysis.

Treatment of the Purified Virus with Detergents

The purified VEE virus was treated in one of the following methods: 1) a mixture of ethyl alcohol and ether (3:1). The preparation of the virus was combined with a mixture of reagents in a 1-10 ratio. The mixture stood for 72 hrs at 4°, then was centrifuged (2500 rpm for 20 min's), the super-suspended fluid vaporized and the fatty stain was emulsified in a physiological solution; 2) a mixture of tween (5 mg/ml of viral suspension) with two units of ether (20 min's at 20° with agitation). The ether was removed by using the centrifuge and by air filtration; 3) nonionic detergents -- 1 % of nonydete P-40 and 2 % of Triton x-100 (30 min's at 20° with agitation); 4) ionic detergents -- 1 % sodium dodecylsulfate (SDS), (30 min's at 20°) and 0.5 % sodium desoxycholate (DOC), (5 min's at 20°). After treating the virus with detergents it was dialyzed against 100 units of 0.01 M phosphate buffer containing 0.1 M NaCl, pH 7.85 for 24 hours at 10°. Infection activity of the VEE virus following the action of the mixture of alcohol and ether was 3-3.5 lg BOE/ml, tween and ether -- 2.0-3/5 lg BOE/ml, nonydete P-40 -- 4-3.5 lg BOE/ml, Triton X-100 -- 3.0-3.5 lg BOE/ml, SDS -- 3.5-4 lg BOE/ml. Serological activity of the antigens subjected to various actions was tested with the complement fixation test [CFT] with specific immune sera. For purposes of control

we used an untreated VEE virus maintained under the same conditions but not treated with detergents, the infectious titre of which was 6-8 lg BOE/ml.

Obtaining Cellular Antigens

Mitochondria fractions were obtained according to standard methodology by the method of differential centrifugation (1). A fraction of cellular juice (S-105) was obtained following centrifugation of the post-mitochondrial supernatant at 105,000 g. This fraction did not contain membrane structures of the cell.

Preparation of the Serum and Formulation of the Reaction

We employed the following immune sera: rabbit serum for chick fibroblasts (FEK), for mitochondria of chick embryo fibroblasts (MFEK), for fractions of cellular juice secreted by the FEK (S-105). The sera were obtained according to a formula previously proposed (6). In order to find viral antigens we used ascitic fluid from mice immunized with a purified VEE virus.

Adsorption of anti-cellular sera (in a 1:10 dilution) was achieved by using erythrocytes of sheep at room temperature for 30 min's. The immune ascitic fluid of the mice was additionally adsorbed by the FEK and in some instances by the tissue of the chorion-allantois membrane of the chick embryo. The adsorbed sera were centrifuged at 105,000 g for 1 hour in order to remove anticomplements.

The CFT was formulated according to the method previously described (7). For purposes of antigens we employed hydro-saline extracts from cells as well as the VEE virus, native and treated with detergents,

as described above. The functional doses of the antigens were arrived at by the method of optimal titration (2-4 units of the antigen).

Group antigens A and H in the photoelectrocolorimeter and in the cellular fractions, as well as in the viral preparations were calculated using human isosera and phytohemagglutinins. We employed the method of selective adsorption of antibodies of normal human serum α and β (correspondingly lecithin) according to the previously described method. Extracts of the Dolichos biflorus L seeds (to calculate antigen A) and Cytisus sessilifolius L. seeds (to calculate antigen H) served as the phytohemagglutinins.

Results

Table 1 presents the results of the investigation of the specie-specific cell antigen of the purified VEE virus. The immune sera obtained with the photoelectrocolorimeter and MFEK resulted in a relatively weak CFT with the native purified virus. However, following treatment of the virus with detergents serological activity of the virus increased 8-16 fold. The immune serum of fraction S-105 containing solluble cell protein provided a negative reaction with the native virus. Following the destruction of the virus by using detergents the reaction became positive pointing to the presence of solluble antigens of cellular origin in the viral composition. Species antigen was also contained in cell mitochondria.

Table 2 shows results of the detection of the Forssman antigen in the virus. It was obvious that the mitochondrial fraction (MEFK) was particularly abundant in the content of the Forssman antigen while native

FEK contained a lesser amount of this antigen. Fraction S-105 obtained from FEK contained almost no Forssman antigen. The Forssman antigen was not found in the purified and concentrated VEE virus. However, following virus treatment with a mixture of alcohol and ether and triton and in particular nonydete we noted an expressed positive complement fixation reaction pointing to the presence in the virus of the heterogenic Forssman antigen.

Thus, these experiments have shown that the Forssman antigen is absent from the surface of native undisturbed VEE virions and is found in a sufficiently active form only following the destruction of the virions using nonydete, triton and a somewhat weaker mixture of alcohol and ether. Following the treatment of the virus with a mixture of tween with ether, SDS or DOC, the Forssman antigen was not present.

We also studied the VEE virus for presence of antigens analogous to human group antigens. As Table 3 shows the native concentrated virus did not bind the agglutinins α or β from the standard human sera. However, following the treatment of the virus with nonydete there clearly appeared the specific ability to bind agglutinins α pointing to the presence of antigen A in the virus. Group antigen A was also found in FEK, in fraction S-105 but in much greater quantities in MFEK.

Analogous results were obtained by us in studying the group antigens in the VEE virus with the help of phytohemagglutinins. For this purpose we employed extracts from the Dolichos biflorus L. seed which made it possible to find the group antigen A_1 and phytohemagglutinins from seeds Cytisus sessilefolius L. in order to calculate antigen H. As

Table 4 shows virus preparations treated with nonydete P-40 cause a noticeable decrease in the hemagglutinin activity of phytohemagglutinin anti-A_1 pointing to the presence in them of group antigen A. The native virus inhibiting the activity in relation to the phytohemagglutinin anti-A_1 did not survive. In analogous experiments using extracts of the Cytisus sessilifolous seeds it was established that the VEE virus purified in a linear gradient of saccharose density and treated with a detergent contains the group antigen H. The FEK in which the virus was cultivated, the MFEK and the S-10$\frac{1}{2}$ fraction also possessed the ability to bind the phytohemagglutinin anti-H. In a native, purified and concentrated VEE virus we did not find the group antigen H.

Discussion

Highly purified and biologically active VEE virus, as our experiments have shown, includes in the structure of its supercapside various specific antigens of the host cell: species-specific, Forssman antigen and group antigens A and H. Antigens of the host cell in the VEE virus are not positioned alike: species antigens are localized mostly on the surface and are accessible to the effects of appropriate antibodies, the Forssman antigens and group antigens are localized in the supercapside of the virion much deeper and the native undestroyed virions are not subject to the effects of specific antibodies.

Our data is not in agreement with the theses that antigens of the host cell are formed as a result of the covering of the virions by fragments of the cell membrane because the Forssman and group antigens which are located on the surface of the cell membrane in native virions

are absent. Apparently, the inclusion of cell antigens in the virion takes place in the process of the gathering of the virion supercapsid in the cell contents on the basis of physico-chemical laws and is not the result of the mechanical covering of the cell membrane virion. Synthesis of these antigens is coded by the cell genome. The cell antigens, apparently, are included in the supercapside structures of the virion in the form of a single glycolipid complex possessing specific properties of group, heterogeneous (Forssman) and species antigens. Burge and Huang (9) proposed the thesis that the initial addition of ordinary cell carbohydrates to the amino acids of the virus protein takes place as a result of the ferment programmed by the virus. The subsequent construction of the carbohydrate chain in the virus is accomplished by cell transferase. This hypothesis, however, does not take into consideration the mechanism of the formation of glycolipid complexes which include the Forssman and group antigens.

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Table 1

SPECIES-SPECIFIC CELL ANTIGEN OF THE VEE VIRUS

Immune serum	Results of the Complement Fixation Test with the Antigen						
	virus				cell		
		Treated with detergent					
	Native	Alcohol + ether	Triton	Nonidet	FEK	MFEK	S-105
Anti-FEK	10	40	80	160	320	80	$\leq$40
Anti-MFEK	20	$\geq$40	160	160	160	640	40
Anti-S-105	--	40	40	40	$<$40	80	160
Anti-VEE	320	160	$>$320	$>$320	--	--	--

Designation -- hemolysis

Note. We cite values of inverse titres of serum activity

Table 2

FORSSMAN ANTIGEN IN THE VEE VIRUS

Subject of Investigation	Results of Complement Fixation Test of the Serum of the Forssman Antigen in Solution					
	1:20	1:40	1:80	1:160	1:320	1:640
Guinea pig kidney native	++++	++++	++++	++++	++++	+++
FEK--native	++	++	+	--	--	--
MFEK--native	++++	++++	++++	++++	+++	++
"S-105"	+	--	--	--	--	--
VEE virus						
treated with nonydete	++++	++++	++	--	--	--
treated with triton	++++	+++	--	--	--	--
treated with ether + alcohol	+++	+	--	--	--	--
treated with SDS	--	--	--	--	--	--
treated with DOX	--	--	--	--	--	--
native	--	--	--	--	--	--

Designations: +, ++, +++, ++++ degree of hemolysis inhibition;

-- hemolysis.

Table 3

GROUP ANTIGEN A IN THE VEE VIRUS

Subject of Investigation	Standard human serum	Standard human erythrocytes	Results of RTGS following adsorption in solution				
			1:2	1:4	1:8	1:16	1:32
Control	α	A	+++	+++	+++	++	+
	β	B	+++	+++	++	++	+
VEE virus	α	A	+++	++	+(+)	+	--
	β	B	+++	+++	+++	++	+(+)
VEE virus + HP-40	α	A	+(+)	--	--	--	--
	β	B	+++	+++	++(+)	++	+(+)
MFEK	α	A	++	--	--	--	--
	β	B	+++	+++	+++	++	+(+)
FEK	α	A	++	+(+)	+	--	--
	β	B	+++	+++	+++	++	+(+)
S-105	α	A	++	+(+)	+	+	--
	β	B	+++	+++	+++	++	++

Designations here and in Table 4: +, ++, +++ degree of hemagglutination;

-- absence of hemagglutination; HP-monyde: P-40.

Table 4

GROUP ANTIGENS A AND H IN THE VEE VIRUS LOCATED WITH THE USE OF SPECIFIC PHYTOHEMAGGLUTININS

Subject of Investigation	Phytohemagglutinin	Results RGA with group phytohemagglutinins				
		1:2	1:4	1:8	1:16	1:32
Control	Anti-A_1	+++	+++	+++	+++	++
VEE virus	"	+++	+++	++	++	--
VEE virus + HP-40	"	++	+	--	--	--
FEK	"	++	++	+	--	--
MFEK	"	++	+	--	--	--
S-105	"	+++	++	+	+	--
Control	Anti-H	+++	+++	+++	++	+(+)
VEE virus	"	+++	++	+	--	--
VEE virus + HP-40	"	++	+(+)	--	--	--
FEK	"	+(+)	+	+	--	--
MFEK	"	--	--	--	--	--
S-105	"	+++	++	++	--	--